Amendments to the Claims

1. (previously presented) A method for differentiating one or more pluripotent embryonic stem (ES) cells comprising:

   a. culturing the ES cells at low density in a serum-free and feeder-layer free media comprising leukemia inhibitory factor; and
   b. allowing said ES cells to differentiate to primitive neural stem cells.

2. (previously presented) The method according to claim 1 for differentiating embryonic stem cells to cells with markers characteristic of neural cells comprising:

   a. culturing the embryonic stem cells in the serum free and feeder-layer free media at low cell density wherein said density is selected to minimize ES cell aggregation or EB formation; and
   b. allowing said cells to differentiate.

3. (previously presented) The method of claim 2 wherein the cell density is selected as to avoid EB formation.

4. (original) The method of claim 1 wherein said cell density is greater than 0 cells/μl to 50 cells/μl.

5. (original) The method of claim 4 wherein the cell density is greater than 0 cells/μl to 20 cells/μl.

6. (original) The method of claim 5 wherein the cell density is greater than 0 cells/μl to 10 cells/μl.

7. (original) The method of claim 6 wherein the cell density is 10 cells/μl.

8. (original) The method of claims 6 wherein there is no EB formation.

9. (previously presented) The method of claim 7 wherein the differentiating ES cells form at least one sphere colony.

10. (previously presented) The method of claim 1 wherein the differentiating ES cells form at least one sphere colony.

11. (original) The method of claim 1 wherein the serum free media further comprises a cytokine.

12. (canceled)

13. (previously presented) The method of claim 1 wherein the primitive neural stem cells are pluripotent.

14. (previously presented) The method of claims 1 wherein the serum free media further comprises a growth factor.

15. (original) The method of claim 14 wherein the growth factor is selected from the members of the fibroblast growth factor (FGF) family of growth factors.

16. (original) The method of claim 15 wherein the growth factor is FGF2.

17. (previously presented) The method according to claim 1 wherein the media comprises Noggin or a compound from the Cerberus family of proteins.

18. (canceled)

19. (canceled)

20. (previously presented) A method for producing secondary primitive neural stem cell colonies comprising:

a. culturing ES cells in low cell density serum-free and

feeder-layer free media comprising leukemia inhibitory factor for a time and under conditions sufficient to differentiate the said ES cells to primary primitive neural stem cell colonies;

b. dissociating and subcloning the primary primitive neural stem cell colonies generated from the said ES cells; and

c. administering a growth factor or survival factor to the dissociated neural cells to produce secondary primitive neural stem cell colonies.

21. (original) A method according to claim 20 wherein the growth factor is selected from among the members of the fibroblast growth factor (FGF) family of growth factors.

22. (original) A method according to claims 21 wherein the growth factor is FGF2.

23. (canceled)

24. (canceled)

25. (previously presented) An isolated primitive neural stem cell expressing one or more neural precursor cell marker and/or one or more neural-specific mRNA molecule, and having multilineage potential.

26. (original) A cell according to claim 25 wherein the neural precursor marker nestin is expressed.

27. (original) A cell according to claim 25 or 26 wherein the neural-specific mRNA molecule is Emx2 or HoxB1.

28. (currently amended) A method according to ~~of~~ claim 1 ~~for~~ further comprising analyzing the role of genes in the

regulation of neural fate specification.

29. (previously presented) An isolated primitive neural stem cell produced by the method of claim 1 that comprises neural cell markers and is pluripotent.

30. (previously presented) An isolated primitive neural stem cell.

31. (previously presented) A method of producing a pre-selected cell type derived from a cell of claim 30 comprising, culturing the cells in media comprising leukemia inhibitory factor under differentiating conditions that promote formation of the cell type.

32. (original) The method of claim 31 wherein the pre-selected cell type is a neural cell, and the differentiating conditions comprise culturing the cell in a serum free media that comprises FGF2.

33. (currently amended) A method for screening for modulators of primitive neural stem cell differentiation comprising:

a. culturing primitive neural stem cells in serum-free and feeder-layer free media comprising leukemia inhibitory factor under low density conditions in the presence of the potential modulator[[;]] under conditions that produce differentiation in the absence of the modulator;
b. detecting any differentiation of the cells and cell types generated, if any, in the presence of the modulator compared to differentiation and cell types generated in the absence of the modulator;
c. determining whether the modulator affects the differentiation of the cells.

34. (original) A method in accordance with claim 33, wherein the modulators comprise any culturing conditions that may modulate cellular differentiation.

35. (currently amended) A method for screening for differentiation factors of cellular development comprising:

a. culturing ~~the~~ pluripotent embryonic stem (ES) cells in serum free media comprising leukemia inhibitory factor at low cell density in the presence of the differentiation factor;
b. allowing cells to differentiate;
c. detecting differentiation of the cells, if any.

36. (currently amended) A method of claim 35 ~~for screening for differentiation factors of~~ further comprising determining whether the differentiation of the cells comprises neural cell development.

37. (previously presented) A method for screening for differentiation factors of cellular development comprising:

a. culturing the cells of claim 29 in serum free media comprising leukemia inhibitory factor, in the presence of the differentiation factor.
b. detecting any differentiation of the cells.

38. (original) The method of claim 37, wherein the media further comprises FGF2.

39. (canceled)

40. (canceled)

41. (currently amended) The method of claim 1 ~~for obtaining~~ further comprising determining whether the cells differentiate into a homogenous uniform cell base.

42. (currently amended) The method of claim 29 ~~wherein the cell base is~~ further comprising determining whether the cells differentiate into a neural cell base.

43. (currently amended) [[A]] The method ~~for supplying cells for transplantation comprising culturing cells pursuant to the method~~ of claim 1 further comprising culturing the cells in a transplantation media.

44. (canceled)

45. (canceled)

46. (canceled)

47. (previously presented) A method for producing secondary primitive neural stem cell colonies comprising:

a. culturing ES cells in low cell density serum-free and feeder-layer free media comprising leukemia inhibitory factor for a time and under conditions sufficient to differentiate the said ES cells to primary primitive neural stem cell colonies;
b. dissociating and subcloning the primary primitive neural stem cell colonies generated from the said ES cells; and
c. administering LIF or B27 to the dissociated neural cells to produce secondary primitive neural stem cell colonies.

48. (previously presented) The primitive neural stem cell of claim 30, wherein the cell is isolated from an embryonic stem cell.

49. (previously presented) An isolated sphere colony

comprising primitive neural stem cells.

50. (canceled)